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SYNTHESIS OF BENZO[c]QUINOLIZIN-3-ONES: SELECTIVE NON-STEROIDAL INHIBITORS OF STEROID 5α-REDUCTASE 1

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Abstract: A short and efficient synthesis of novel benzo[c]quinolizin-3-one derivatives is described. The synthesis is based on the tandem Mannich-Michael cyclization between 2-silyloxy-1,3-butadienes and a *N-t*-Boc iminium ion. The prepared derivatives are selective inhibitors of human steroid 5α-reductase isoenzyme 1, thus having potential application as drugs for treatment of male pattern baldness and other DHT-dependent skin disorders.

Steroid 5α-reductase is a family of two isozymes, named type 1 (5αR-1) and type 2 (5αR-2), that catalyzes the NADPH-dependent reduction of testosterone (T) to dihydrotestosterone (DHT), and other 3-oxo-4-ene steroids (e.g., progesterone, corticosterone, etc.) to the corresponding 5α compounds. The 5α-reductase and its product DHT play an important role in the pathogenesis of some important human diseases, i.e. benign prostatic hyperplasia (BHP) and prostatic cancer, and skin disorders such as acne, alopecia, pattern baldness in men and hirsutism in women. Thus, the discovery of potent and selective inhibitors for the two isozymes appears of great importance for the pharmacological treatment of these pathologies.[1] Although the precise role of each 5αR isoenzyme in DHT-dependent diseases is yet to be fully elucidated, however it is now clear that inhibitors of both enzymes are best applied to the BPH treatment, whilst selective inhibitors of 5αR-1 could potentially be used for skin disorders treatment.

As part of our studies on 5α-reductase[2] and its inhibitors[3] we discovered a series of 19-nor-10-azasteroids **1** (Figure 1) which were potent inhibitors of both human 5αR isoenzymes.[4]

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In designing these inhibitors (Figure 1), the vinylogous amide was inserted into the steroid nucleus as substrate-like transition state mimic of the T to DHT conversion.[4,5] The presence of the nitrogen atom at position 10, conjugated with the carbonyl at C-3, is an essential feature for a good inhibition since, providing an increase of the negative partial charge on the oxygen,[4,5] it probably determines a strong interaction with an electrophilic residue in the enzyme active site.

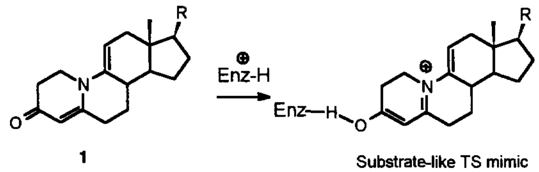


Figure 1.

Recently, some non-steroidal 5αR inhibitors have been designed starting from the related parent steroidal inhibitors, and some of them have been found to be potent and selective 5αR-1 inhibitors.[1] Selected examples of these non-steroidal inhibitors and their corresponding steroidal parents are shown in Figure 2.

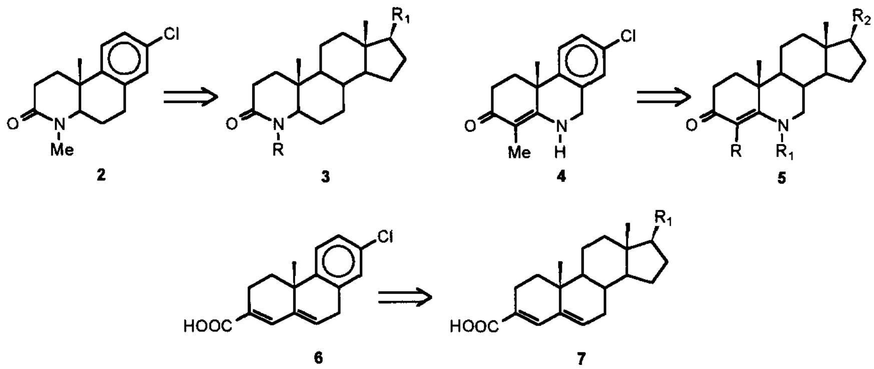
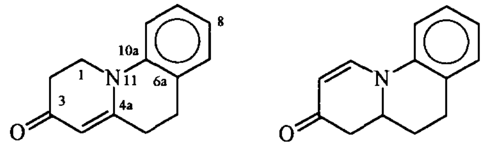


Figure 2.

Thus, benzoquinoline derivatives (typified by compound **2**) were derived from 4-azasteroids **3**, phenantridine derivatives (typified by compound **4**) from 6-azasteroids **5**, and diene acids (typified by compound **6**) from steroidal acrylates **7**.[1]

Prompted by these reports, we targeted two types of novel benzo[c]quinolizin-3-ones **8** and **9** (Figure 3) which, maintaining the typical enaminone moiety of 19-nor-10-azasteroids **1**, are differentiated by the position of the conjugated C-C double bond. Compound **8**, having the unsaturation at C-4—C-4a, belongs to the *1H*-benzo[c]quinolizines series and was designed as a potential substrate-like transition state mimic, whereas compound **9**, of the 4a*H*-benzo[c]quinolizines series, was designed as a potential product-like transition state mimic.

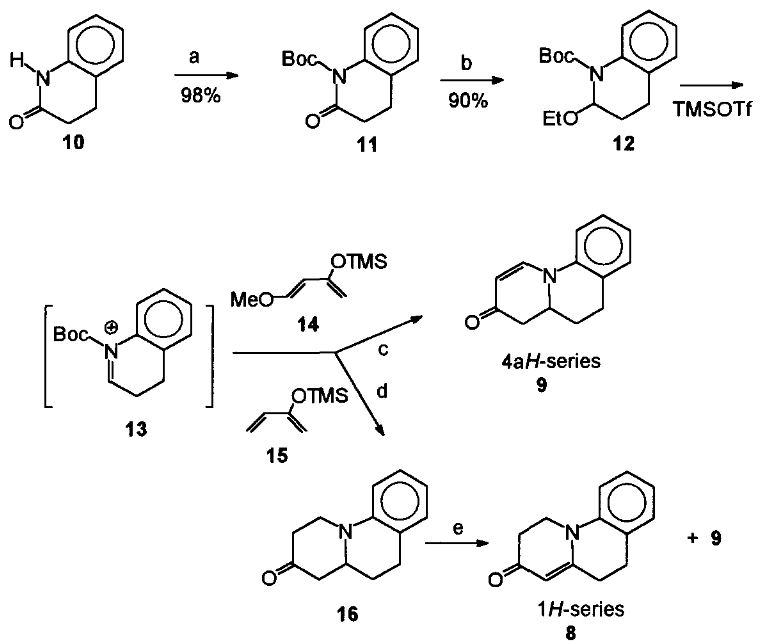


1*H* series
8

4a*H* series
9

Figure 3.

We report herein on a short and efficient 4-step synthesis of novel benzo[c]quinolizine compounds **8** and **9** (Scheme 1), and their inhibition against human recombinant 5αR-1 and human prostate homogenates (which contain mainly the type 2 isoenzyme).[4]



Scheme 1.
Reagents and conditions: a) Boc_2O, Et_3N, DMAP (cat.) CH_2Cl_2, 16 h, 25 °C; b) $NaBH_4$ in EtOH, -25°C, 4 h; then HCl 2N in EtOH, pH 3-4, 0 °C, 1.5 h; c) Danishefsky's diene **14**, Et_3N, TMSOTf in CH_2Cl_2, 0 → 25 °C, 30 min; then $NaHCO_3$ (sat) 36 h; d) 2-silyloxy-1,3-butadiene **15**, Et_3N, TMSOTf in CH_2Cl_2, 0 → 25 °C, 30 min; then $NaHCO_3$ (sat) 36 h; e) $Hg(OAc)_2$, EDTA tetrasodium salt, 5% CH_3COOH (aq), 90 °C, 2 h.

The dihydroquinolin-2-one **10**[6] (commercially available) was protected as *N*-*t*-Boc, then reduced to the ethoxy derivative **12** by treatment with $NaBH_4$ in EtOH at -25°C followed by the slow addition of HCl 2N up to pH 3-4, according to a reported method.[7] The key step of the synthesis was the Lewis acid catalyzed tandem

Mannich-Michael cyclization of *N-t*-Boc iminium ion **13** with a silyloxydiene. The generation *in situ* of the *N-t*-Boc iminium ions from *N-t*-Boc α-ethoxy derivatives such as **12**, can be promoted by different Lewis acid,[8] but in our hand the best choice was the use of TMSOTf according to the methodology reported by Pilli *et al.*[7] We have recently extended a similar strategy to an efficient short synthesis of 19-nor-10-azasteroids.[9] In the synthesis of the title compounds, the use of Danishefsky's diene **14** led directly to 4a*H*-benzo[c]quinolizin-3-one **9** in fair yield (30%).[10] Saturated compounds **16** was instead obtained by reaction of **13** with 2-silyloxy-1,3-butadiene **15** (prepared as reported[11] or generated *in situ* from methyl vinyl ketone, TMSOTf and Et_3N). The introduction of the double bond at the 4-4a position was achieved by $Hg(OAc)_2$ oxidation,[12] leading to target 1*H* compound **8** in 20% yield,[10] besides a minor amount (ca. 10%) of the corresponding 4a*H* derivative **9**.

Compounds **8** and **9** were tested, in comparison with the know inhibitor finasteride, against human recombinant 5αR-1 and homogenates of human prostate according to reported procedures.[4,13] The resulting IC_{50} values (shown in Table 1) indicate that these compounds were selective inhibitors of 5αR-1, with a potency dependent on the A-ring unsaturation, whereas no inhibition was observed against homogenates of human prostate. This selectivity was instead not observed for the parent 19-nor-10-azasteroids:[4] for example, the most potent azasteroid **1**, with R = β-CONHBu^t (Figure 1), was a dual inhibitor, displaying almost identical inhibition potency toward the two isoenzymes (Table 1). In analogy to the parent 19-nor-10-azasteroids, preliminary experiments shown that these benzo[c]quinolizinones were reversible 5α-R inhibitors.

Table 1. Inhibition against Human 5α-Reductase 1 and 2

Compound	Inhibition K_i (nM)		Compound	Inhibition IC_{50} (nM)	
	5αR-1	5αR-2		5αR-1[a]	5αR-2[b]
2[14]	9	>1 000	**8**	298 ± 75	ni[c]
4[15]	920	20 000	**9**	5130 ± 130	ni[c]
6[16]	1200	260	**1**[d]	127 ± 12[e]	122 ± 37[b]
			Finasteride	911 ± 85	1.2 ± 0.25

[a]Determined on recombinant CHO cells.[13] [b]Determined on human prostate homogenates.[4] [c]No inhibition observed up to a 10 μM concentration of inhibitor. At this concentration the inhibition was 17% for **8** and 11% for **9**. [d]9:1 mixture of $\Delta^{9(11)}$ and $\Delta^{8(9)}$ isomers. R=β-CONHBu^t. [e]Determined on DU-145 cells.

Of the two compounds, the best inhibitor was 2,3,5,6-tetrahydro-1*H*-benzo[c]quinolizin-3-one **8** which, if compared to the other non-steroidal inhibitors above mentioned (see Figure 2), seems to behave more similarly to compound **2** (LY266111) than to compound **4**, which was a selective but weaker 5αR-1 inhibitor, or **6**, which was more potent against 5αR-2 than 5αR-1. As expected, in the same experiments finasteride was a more potent 5αR-2 inhibitor, but a weaker 5αR-1 inhibitor in comparison to **8**. The inhibition potency of the 4a*H* derivative **9** was lower than that of 1*H* derivative **8**, in agreement with an analogous observation made on

19-nor-10-azasteroids.[4] The observed selective inhibition indicates a potential application of this new type of non-steroidal inhibitors as drugs for treatment of male pattern baldness and other DHT-dependent skin disorders.[1] Studies aimed at increasing the potency of these compounds by introducing different substituents on the benzo[c]quinolizin-3-one skeleton are in progress.

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